

APPENDIX II

1. (twice amended) A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, [wherein said antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity] which is murine monoclonal antibody 33.28 as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413.

[2. An antibody according to claim 1 which is mouse monoclonal antibody 33.28 (ATCC HB-12315) or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 3328.]

3. (twice amended) An antibody according to claim [2] 1 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 61.1 kilodaltons as measured by gradient polyacrylamide gel electrophoresis.

4. (amended) [An antibody according claim 1] A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, which is mouse monoclonal antibody 31.1, as produced by hybridoma PCA 31.1 [(ATCC HB-12314)] , deposited with the American Type Culture Collection and assigned accession number PTA-2497[or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 31.1].

5. (three times amended) [An] A monoclonal antibody [according to claim 4 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 72 kilodaltons] which comprises an antigen-binding region derived from the H chain of a murine monoclonal antibody 33.28 as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413.

6. (three times amended) An antibody according to claim [2] 1 wherein said colon carcinoma-associated antigen is a glycoprotein, the protein component having a molecular weight of 61.1 kilodaltons as measured by gradient polyacrylamide gel electrophoresis.

7. (twice amended) An antibody according to claim 1, 4 or 5 immobilized on a solid phase.

8. (twice amended) An antibody according to claim 1, 4 or 5 which is detectably labeled.

10. (twice amended) An antibody according to claim 1, 4 or 5 conjugated to a cytotoxic radionuclide.

11. (twice amended) An antibody according to claim 1, 4 or 5 conjugated to a cytotoxic drug.

12. (twice amended) An antibody according to claim 1, 4 or 5 conjugated to a cytotoxic protein.

[16. A monoclonal antibody against the monoclonal antibody of claim 1.]

[17. A monoclonal antibody against the monoclonal antibody of claim 2.]

[18. A monoclonal antibody against the monoclonal antibody of claim 3.]

[19. A monoclonal antibody against the monoclonal antibody of claim 4.]

[20. A monoclonal antibody against the monoclonal antibody of claim 5.]

[21. A monoclonal antibody against the monoclonal antibody of claim 6.]

22. (twice amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 33.28 [(ATCC HB-12315)] as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413, in a sample comprising:

(a) contacting said sample with an effective binding amount of the antibody according to claim 1; and

(b) detecting said antigen by detecting the binding of the antibody to the [purified] colon carcinoma _associated protein antigen.

23. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 31.1 [(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497, in a sample comprising:

(a) contacting said sample with an effective binding amount of the antibody according to claim [1] 4 or claim 5; and

(b) detecting said antigen by detecting the binding of the antibody to the [purified] colon carcinoma _associated protein antigen.

24. (amended) A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having a colon cancer;

(b) contacting the specimen with monoclonal antibody 33.28 [(ATCC HB-12315)] , as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413;

(c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex by the stain.

25. (amended) A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having colon[-] carcinoma;

(b) contacting the specimen with mouse monoclonal antibody 31.1 [(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497;

(c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex.

28. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

(a) mouse monoclonal antibody 31.1[(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497;

(b) reagents for immunoperoxidase and secondary antibody;

(c) immunoperoxidase; and

(d) colorizing reagents.

29. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

(a) mouse monoclonal antibody 33.28 [(ATCC HB-12315)] , as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413;

(b) reagents for immunoperoxidase and secondary antibody;

- (c) immunoperoxidase; and
- (d) colorizing reagents.

[30. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

- (b) said antigen is not detectable on normal colon cancer free human tissues;

- (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

- (d) said antigen is specifically immunogenic in humans; and

- (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain an antibody to said antigen or an active component thereof, and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.]

[31. A kit according to claim 30 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.]

[32. A kit according to claim 30 wherein the reporter molecule is an enzyme.]

[33. A kit according to claim 30 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.]

[34. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain monoclonal antibody 31.1 (ATCC HB-12314) to said antigen and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.]

[35. A kit according to claim 34 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.]

[36. A kit according to claim 32 wherein the reporter molecule is an enzyme.]

[37. A kit according to claim 33 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.]

[38. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,
said kit comprising a first container adapted to contain monoclonal antibody 33.28 (ATCC HB-12315) to said antigen and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.]

[39. A kit according to claim 38 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.]

[40. A kit according to claim 38 wherein the reporter molecule is an enzyme.]

[41. A kit according to claim 38 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.]

[42. The monoclonal antibody of claim 1 which is a chimeric antibody.]

43. (amended) [The] A chimeric antibody [according to claim 42] which is a chimeric mouse/human antibody Chi #1 as produced by the cell line deposited with the American Type Culture Collection and assigned accession number [(ATCC) CRL-12316[]].

[44. The chimeric antibody according to claim 42 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of 72 kilodalton.]

45. (amended) A composition comprising the chimeric antibody according to claim [42] 43 in combination with a pharmaceutically acceptable carrier.

[46. A monoclonal antibody against the chimeric antibody of claim 42.]

47. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to the mouse/human chimeric antibody Chi #1 as produced by the cell line deposited with the American Type Culture Collection and assigned accession number [(ATCC) CRL-12316] of claim 42] in a sample comprising:

- (a) contacting said sample with the Chi #1 antibody [according to claim 42]; and
- (b) detecting said antigen by detecting the binding of said antibody to the [purified] colon carcinoma _ associated protein antigen.

48. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon carcinoma;
- (b) contacting the specimen with a chimeric antibody [which binds to an antigen] according to claim [1] 43;
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

49. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon carcinoma;
- (b) contacting the specimen with mouse/human chimeric antibody which binds to an antigen which binds to mouse/human chimeric antibody Chi #1 [(ATCC) as produced by the cell line deposited with the American Type Culture Collection and assigned accession number CRL-12316];
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

50. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse/human chimeric antibody Chi #1 (ATCC CRL-12316);
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

APPENDIX III

1. (twice amended) A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, [wherein said antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity] which is murine monoclonal antibody 33.28 as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413.

[2. An antibody according to claim 1 which is mouse monoclonal antibody 33.28 (ATCC HB-12315) or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 3328.]

3. (twice amended) An antibody according to claim [2] 1 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 61.1 kilodaltons as measured by gradient polyacrylamide gel electrophoresis.

4. (amended) [An antibody according claim 1] A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, which is mouse monoclonal antibody 31.1, as produced by hybridoma PCA 31.1 [(ATCC HB-12314)] , deposited with the American Type Culture Collection and assigned accession number PTA-2497[or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 31.1].

5. (three times amended) [An] A monoclonal antibody [according to claim 4 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 72 kilodaltons] which comprises an antigen-binding region derived from the H chain of a murine monoclonal antibody 33.28 as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413.

6. (three times amended) An antibody according to claim [2] 1 wherein said colon carcinoma-associated antigen is a glycoprotein, the protein component having a molecular weight of 61.1 kilodaltons as measured by gradient polyacrylamide gel electrophoresis.

7. (twice amended) An antibody according to claim 1, 4 or 5 immobilized on a solid phase.

8. (twice amended) An antibody according to claim 1, 4 or 5 which is detectably labeled.

9. (original) An antibody according to claim 8 wherein said detectable label is a radiolabel.

10. (twice amended) An antibody according to claim 1, 4 or 5 conjugated to a cytotoxic radionuclide.

11. (twice amended) An antibody according to claim 1, 4 or 5 conjugated to a cytotoxic drug.

12. (twice amended) An antibody according to claim 1, 4 or 5 conjugated to a cytotoxic protein.

13. (original) A composition comprising an antibody according to claim 10 in combination with a pharmaceutically acceptable carrier.

14. (original) A composition comprising an antibody according to claim 11 in combination with a pharmaceutically acceptable carrier.

15. (original) A composition comprising an antibody according to claim 12 in combination with a pharmaceutically acceptable carrier.

[16. A monoclonal antibody against the monoclonal antibody of claim 1.]

[17. A monoclonal antibody against the monoclonal antibody of claim 2.]

[18. A monoclonal antibody against the monoclonal antibody of claim 3.]

[19. A monoclonal antibody against the monoclonal antibody of claim 4.]

[20. A monoclonal antibody against the monoclonal antibody of claim 5.]

[21. A monoclonal antibody against the monoclonal antibody of claim 6.]

22. (twice amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 33.28 [(ATCC HB-12315)] as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413, in a sample comprising:

(a) contacting said sample with an effective binding amount of the antibody according to claim 1; and

(b) detecting said antigen by detecting the binding of the antibody to the [purified] colon carcinoma - associated protein antigen.

23. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 31.1 [(ATCC HB-12314)] as produced by

hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497, in a sample comprising:

- (a) contacting said sample with an effective binding amount of the antibody according to claim [1] 4 or claim 5; and
- (b) detecting said antigen by detecting the binding of the antibody to the [purified] colon carcinoma - associated protein antigen.

24. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon cancer;
- (b) contacting the specimen with monoclonal antibody 33.28 [(ATCC HB-12315)] , as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413;
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

25. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having colon[-] carcinoma;
- (b) contacting the specimen with mouse monoclonal antibody 31.1 [(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497);
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex.

26. (original) A method according to claim 24 wherein the stain is an avidin-biotin immunoperoxidase stain.

27. (original) A method according to claim 25 wherein the stain is an avidin-biotin immunoperoxidase stain.

28. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

(a) mouse monoclonal antibody 31.1[(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497;

(b) reagents for immunoperoxidase and secondary antibody;

(c) immunoperoxidase; and

(d) colorizing reagents.

29. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

(a) mouse monoclonal antibody 33.28 [(ATCC HB-12315)] , as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413;

(b) reagents for immunoperoxidase and secondary antibody;

(c) immunoperoxidase; and

(d) colorizing reagents.

[30. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain an antibody to said antigen or an active component thereof, and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.]

[31. A kit according to claim 30 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.]

[32. A kit according to claim 30 wherein the reporter molecule is an enzyme.]

[33. A kit according to claim 30 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.]

[34. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,

said kit comprising a first container adapted to contain monoclonal antibody 31.1 (ATCC HB-12314) to said antigen and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.]

[35. A kit according to claim 34 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.]

[36. A kit according to claim 32 wherein the reporter molecule is an enzyme.]

[37. A kit according to claim 33 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.]

[38. A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, wherein the antigen has the following characteristics:

- (a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
 - (b) said antigen is not detectable on normal colon cancer free human tissues;
 - (c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
 - (d) said antigen is specifically immunogenic in humans; and
 - (e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,
- said kit comprising a first container adapted to contain monoclonal antibody 33.28 (ATCC HB-12315) to said antigen and a second container adapted to contain a second antibody to said antigen or an active component thereof, said second antibody being labeled with a reporter molecule capable of giving a detectable signal.]

[39. A kit according to claim 38 wherein the reporter molecule is a radioisotope, an enzyme, a fluorescent molecule, a chemiluminescent molecule or a bioluminescent molecule.]

[40. A kit according to claim 38 wherein the reporter molecule is an enzyme.]

[41. A kit according to claim 38 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.]

[42. The monoclonal antibody of claim 1 which is a chimeric antibody.]

43. (amended) [The] A chimeric antibody [according to claim 42] which is a chimeric mouse/human antibody Chi #1 as produced by the cell line deposited with the American Type Culture Collection and assigned accession number [(ATCC) CRL-12316[]].

[44. The chimeric antibody according to claim 42 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of 72 kilodalton.]

45. (amended) A composition comprising the chimeric antibody according to claim [42] 43 in combination with a pharmaceutically acceptable carrier.

[46. A monoclonal antibody against the chimeric antibody of claim 42.]

47. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to the mouse/human chimeric antibody Chi #1 as produced by the cell line deposited with the American Type Culture Collection and assigned accession number [(ATCC) CRL-12316[]] of claim 42] in a sample comprising:

(a) contacting said sample with the Chi #1 antibody [according to claim 42]; and
(b) detecting said antigen by detecting the binding of said antibody to the [purified] colon carcinoma - associated protein antigen.

48. (amended) A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having a colon carcinoma;
(b) contacting the specimen with a chimeric antibody [which binds to an antigen] according to claim [1] 43;

- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

49. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon carcinoma;
- (b) contacting the specimen with mouse/human chimeric antibody which binds to an antigen which binds to mouse/human chimeric antibody Chi #1 [(ATCC) as produced by the cell line deposited with the American Type Culture Collection and assigned accession number CRL-12316[]];
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

50. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse/human chimeric antibody Chi #1 (ATCC CRL-12316);
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.